New Corn Technology:

Scientists Are All Eyes and Ears

When it comes to finding fungus on corn, there's more to it than meets the eye. For years, corn inspectors have used ultraviolet light—under which corn fungus glows a bright greenish-yellow—to search for contaminated kernels. But sifting through piles of harvested corn by hand is a painfully slow—if effective—way to rid the nation's corn crop of unhealthful microbes. So scientists at the U.S. Department of Agriculture (USDA) have developed a new approach: rather than simply looking for fungal contamination, now they're listening for it, too.

In a new twist on an old technique called photoacoustics, a research team led by biochemist Richard Greene at the USDA's National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, has found that contaminated corn emits a telltale "ping" sound when heated and then quickly cooled. When recorded by a computer, this sickly sound can be separated from the tones produced by healthy corn. It's an offbeat strategy for corn inspection—and it could be the prelude to a whole new way of checking grain quality worldwide. "I think we've tapped into a powerful approach," Greene says.

Contaminated corn is a perennial problem worldwide. Aside from hungry insects and fickle weather, corn crops routinely endure the onslaught of *Aspergillus flavus*, a fungus that burrows inside and grows on the surface of corn kernels. *A. flavus* gives off a carcinogenic by-product called aflatoxin that some researchers blame for high rates of liver cancer in Asia and Africa, where rice and corn are food staples. Aflatoxin can also cause hepatitis, cirrhosis, and death. Acute aflatoxin poisonings have been recorded, such as one in 1974 in western India in which roughly 100 people died and several hundred others got sick from eating moldy corn. Less severe exposure events happen fairly frequently in these regions.

In the United States, aflatoxin's major threat is to farm animals, which can get sick or even die from consuming too much of the toxin. *A. flavus* also attacks harvested peanut, cotton, and tree nut crops, as well as milk stores, particularly in the southern United States, where the weather can be hot and dry for months at a time. When the weather is hot and dry, crop plants can become stressed and therefore more susceptible to invasion by *A. flavus*. The fungus usually sets in while crops are growing, but can also stow away on corn undetected and begin to give off aflatoxin while the kernels are being stored in silos. Metabolites of aflatoxin show up in the milk of dairy cows that eat contaminated feed. Consumers are protected by milk testing, but farmers—who can lose big money with unproductive cows—must monitor the feed their cows eat to prevent aflatoxin poisoning of the animals themselves. According to USDA regulation, crops sold for animal feed are allowed to harbor no more than 20 contaminated parts of corn per billion. According to estimates by Walter Morrison, an agronomist at Louisiana State University in Baton Rouge, Louisiana corn inspectors rejected up to a quarter of the corn grown during drought conditions that arrived at grain elevators for a first pass last year. In the Midwest—home to most of the cornfields in the United States—a serious dry spell can mean hundreds of millions of dollars in crops lost to aflatoxin.

The Song of the Corn

Inspecting corn is a low-tech, labor-intensive endeavor. Traditionally, grain inspectors test random corn samples by hand by shining a bright greenish-yellow fluorescent (BGYF) light on kernels. If the kernels are contaminated with fungus, they turn a vivid greenish-yellow. Suspicious samples are then tested further in the lab.

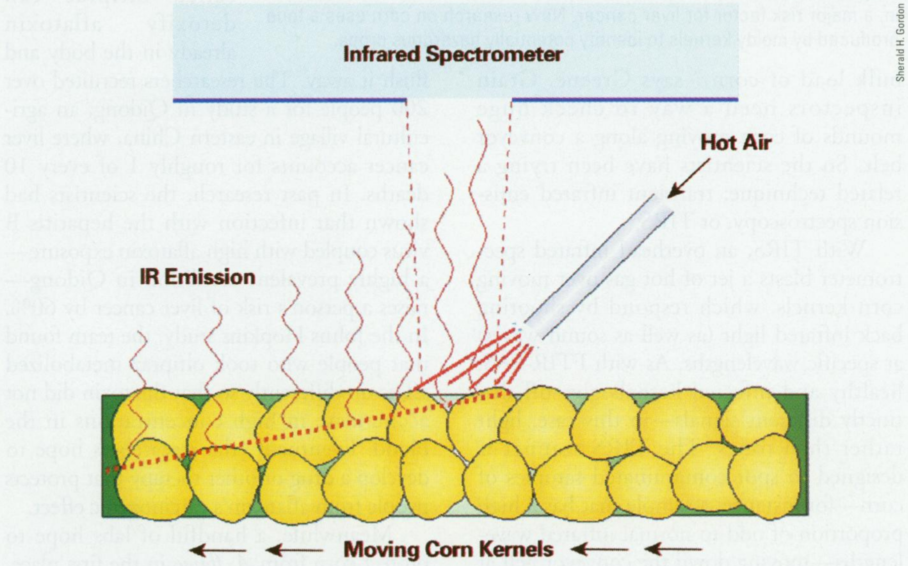
While the BGYF test works fairly well, identifying about 85% of infected kernels, Greene thought inspectors could do better. Hunting for a new technique, he stumbled upon an old one: photoacoustics. First discovered by Alexander Graham Bell 120 years ago, photoacoustics is the use of light to generate sound. When solids (in this case, corn kernels) absorb light, they get hotter, warming the surrounding air and boosting air pressure. Then, if the light is quickly blocked, those solids cool. The plummeting air pressure makes a sound like a musical tone. Scientists know that thick solids sound different from thin ones.

The question that arose was whether a computer could be used to distinguish the photoacoustic songs sung by healthy, whole corn kernels from those riddled with fungal holes. While Greene believed the answer was yes, his colleague, NCAUR chemist Sherald Gordon, wasn't so sure. "The fungi are so small and in such a small concentration that I never thought we'd be able to pick [their sound] up," Gordon says.

To test their idea, Greene and Gordon piled plastic bags full of healthy and aflatoxin-tainted corn into a car and drove to a lab at Iowa State University in Ames headed by physicist and photoacoustic spectroscopy expert John McClelland. Using a technique called Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS), light of a certain wavelength was shone on the surface of the corn kernels. The kernels absorbed the light energy and then bounced it back in the form of a sound wave (inaudible to humans). The sound waves were then analyzed by a software program designed by Bruce Wheeler, a computer engineer at the University of Illinois at Urbana–Champaign. Wheeler's program used a neural network—a system that recognizes patterns, much like neurons in the human brain do—to convert the bings and pops of the corn kernels into a numerical analysis. Infected corn proved to create different numerical patterns than healthy corn. The neural network recognized these pattern differences and indicated when a kernel was infected. Thus, the team was thrilled to find that a computer could, indeed, pick up the inaudible songs of the corn and tell healthy kernels from sick ones.

The technique works, Gordon says, because *A. flavus* bores tunnels into the surface of corn kernels, rather like a worm. A pockmarked kernel will absorb and give off heat differently than a solid, healthy one, resulting in a sound wave that is emitted on a different wavelength. In further tests, a computer endowed with a neural network that records the sound waves successfully identified about 96% of contaminated corn—10% more than the traditional BGYF test.

That breakthrough was only the prelude. Because FTIR-PAS works on single kernels, scientists might use it to test suspicious handfuls of corn in the lab. "But you'd go crazy trying this technique with a



Pop corn. Photoacoustic spectroscopy uses a combination of hot air and infrared light to heat corn kernels, which emit different tones (sound waves of certain wavelengths) when they cool depending on whether they are healthy or contaminated with fungus. Contaminated kernels are then identified by a computer program that analyzes the tones.

Crop chorus? Mold on crops such as peanuts and corn produces aflatoxin, a major risk factor for liver cancer. New research on corn uses a tone produced by moldy kernels to identify potentially hazardous crops.

bulk load of corn," says Greene. Grain inspectors need a way to check huge mounds of corn moving along a conveyor belt. So the scientists have been trying a related technique: transient infrared emission spectroscopy, or TIRS.

With TIRS, an overhead infrared spectrometer blasts a jet of hot gas over moving corn kernels, which respond by shooting back infrared light (as well as sound waves) at specific wavelengths. As with FTIR-PAS, healthy and infected kernels give off distinctly different signals—in this case, light rather than tones. The TIRS scanner is designed to spot contaminated samples of corn—for instance, a sample that has a high proportion of odd to normal infrared wavelengths—moving down the conveyor belt at roughly 80 feet per second. The secret, McClelland says, is zeroing in on a narrow region that's deep enough inside the kernel to get reliable measures but thin enough to analyze. Both FTIR-PAS and TIRS can isolate a region inside the corn kernel between its surface and a certain depth that is suitable for chemical analysis. The heat permeates into a precise layer of the corn kernel, which responds by giving off measurable wavelengths of infrared light. "Most solid materials have to be ground, diluted, or squeezed into a thin film to study them up close," McClelland says. "Our technique is notable because you don't have to prepare the sample at all. You just measure it."

Industry observers are keen to see whether TIRS evolves from idea to practical tool. "When it comes to food safety, a technology that's high-throughput and low-cost is a real contribution," says Darlene Solomon, manager of chemical and biological systems at Hewlett-Packard Laboratories in Palo Alto, California. "So a screening technique like this could be quite useful." At the moment, the TIRS technology is only in prototype form and awaits corporate or government funding for further study.

Kernels of Truth

Some scientists hope to go one better than screening for aflatoxin—they want to stop the poison in its tracks. A team of environmental health scientists at The Johns Hopkins University in Baltimore, Maryland, reported in the 17 February 1999 issue of the *Journal of the National Cancer Institute* that a drug called oltipraz can detoxify aflatoxin already in the body and flush it away. The researchers recruited over 200 people for a study in Qidong, an agricultural village in eastern China, where liver cancer accounts for roughly 1 of every 10 deaths. In past research, the scientists had shown that infection with the hepatitis B virus coupled with high aflatoxin exposure—a highly prevalent condition in Qidong—raises a person's risk of liver cancer by 60%. In the Johns Hopkins study, the team found that people who took oltipraz metabolized aflatoxin differently so that the toxin did not accumulate in high concentrations in the blood. Eventually, the researchers hope to develop a drug or other therapy that protects people from aflatoxin's carcinogenic effect.

Meanwhile, a handful of labs hope to protect corn from *A. flavus* in the first place. Since 1991, scientists at the USDA's Agricultural Research Service (ARS) in New Orleans have been testing strains of corn to find those that are resistant to the fungus. Some corn kernels have a protective waxy coat, for example, or carry high levels of proteins that hamper the growth of *A. flavus*. "Corn has a lot of genetic diversity, so we've found a number of sources of resistance," says ARS plant pathologist Robert Brown. He and his colleagues hope to isolate fungus-resistance traits that could be genetically engineered or classically bred into corn strains sold to farmers.

And this summer at the University of Illinois at Urbana–Champaign, plant pathologist Don White and plant physiologist Jack Widholm grew plots of different corn lines that resist aflatoxin. One promising corn line naturally contains enzymes that may break down the walls of *A. flavus* cells. Genes for two enzymes that should also affect *A. flavus* cell walls have also been inserted into other lines. Field test results will show how well the corn stands up against the fungus in the wild.

It's unclear whether the recent furor over genetic engineering of corn might affect these efforts, however. Reporting in the 20 May 1999 issue of *Nature*, entomologists at Cornell University in Ithaca, New York, showed that a common corn engineered to stave off corn borers also inadvertently kills monarch butterflies that come in contact with the corn's pollen. That finding—along with the recent European outcry over genetically engineered foods—leaves some researchers questioning the technology's acceptance, at least in the short term.

For the moment, screening corn for fungi is the only strategy food inspectors have. And new techniques couldn't come at a better time, Widholm says. European governments are pressing the United States to improve the quality of grain exports, even below the 20-parts-per-billion norm for aflatoxin. Photoacoustics could play a role in achieving even safer levels. In addition to finding fungi, Greene says, the TIRS technique can test general grain quality before it's shipped overseas. He suggests that TIRS will supplement, rather than replace, today's inspection techniques. Together, Greene says, the BGYF and TIRS tests could clear out virtually all aflatoxin from the nation's corn, and that's music to his ears.

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Suggested Reading

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